Keller, R., "Ueber den Farbenwechsel des Chamaeleons und einiger anderer Reptilien," *Arch. ges. Physiol.*, **61**, 123-168 (1895).

Lundstrom, H. M., and Bard, P., "Hypophysial Control of Cutaneous Pigmentation in an Elasmobranch Fish," *Biol. Bull.*, **62**, 1-9 (1932).

Mills, S. M., "The Double Innervation of Fish Melanophores," *Jour. Exp. Zoöl.*, **64**, 231-244 (1932).

Parker, G. H., "Cellular Transfer of Substances, Especially Neurohumors," *Jour. Exper. Biol.*, **11**, 81-88 (1934a).

Parker, G. H., "Oil-Soluble and Water-Soluble Neurohumors," *Anat. Rec.*, **60**, Suppl. 30 (1934b).

Parker, G. H., "The Prolonged Activity of Momentarily Stimulated Nerves," *Proc. Nat. Acad. Sci.*, **20**, 306-310 (1934c).

Parker, G. H., Brown, F. A., Jr., and Odiorne, J. M., "The Relation of the Eyes to Chromatophoral Activities," *Proc. Amer. Acad. Arts Sci.*, **69**, 439-462 (1935).

Parker, G. H., and Porter, H., "Regeneration of Chromatophore Nerves," *Jour. Exper. Zoöl.*, **66**, 303-309 (1933).

Parker, G. H., and Porter, H., "The Control of the Dermal Melanophores in Elasmobranch Fishes," *Biol. Bull.*, **66**, 30-37 (1934).

Smith, D. C., "The Action of Certain Autonomic Drugs upon the Pigmentary Responses of Fundulus," *Jour. Exp. Zoöl.*, **58**, 423-453 (1931).

Wyman, L. C., "Blood and Nerve as Controlling Agents in the Movements of Melanophores," *Jour. Exper. Zoöl.*, **39**, 73-131 (1924).

# *THE GROWTH HORMONE AND THE DWARF TYPE OF GROWTH IN CORN*

BY J. VAN OVERBEEK

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated April 6, 1935

1. *Introduction.*—In this paper is given a short report of an analysis of the causes of one of the dwarf types of growth in corn. A more detailed report will be published later. The dwarf type investigated is *nana*,[1] which differs from the normal race by one single gene. The seeds were obtained from Dr. E. G. Anderson, of the California Institute, and were the result of a cross between $F_1$ (*naNA*) with *na na*. As *na* is recessive the plants from these seeds were 50% dwarfs and 50% normals. The difference between nana and normal consists in an inhibition of the growth in length in nana. Even in seedlings, especially when grown in the dark, nana can be distinguished from normal by its shorter length. This difference in growth is caused by a growth inhibition of the mesocotyl of nana. *The length of the coleoptile is the same in both races* (Fig. 1).

The seeds were soaked for several hours, then set out in moist sand and grown in a dark room at constant temperature (24°C.) and constant humidity (90%). The dark room was illuminated with orange light. A Corning filter No. 348 was used, light from which produced no phototropic curvature in corn or in Avena. Four to six days after the seeds were set out, the plants were ready to be used for the experiments. The primary leaf had not then broken through; the length of coleoptile and mesocotyl can be seen in figure 1.

When cells of normal mesocotyls (epidermis) are compared with those in nana, the length of the normals is much the greater. The growth inhibition in nana therefore is a matter of decreased extension.

It has become clear in recent times that there is a hormone in plants which controls the longitudinal growth. This hormone is known as the *growth hormone, growth substance* or *auxin.* Without growth hormone no growth will occur, as F. W. Went (1928) proved.

2. *Less Auxin Given Off by Nana Than by Normal.*—In young corn seedlings the growth substance is produced in the tip of the coleoptile. If we cut off such tips and place them on blocks of 3% agar, the auxin will diffuse into the blocks. The auxin in the blocks can be analyzed by means of a test method designed by Went (1928) and further improved by Van der Wey (1931). From etiolated oat (Avena) seedlings the coleoptile tip is cut off and on the remaining stump a block of agar containing auxin is placed on one side. The auxin will diffuse out of the block and travel down on the side of the plant to which the block is applied. This causes a greater growth on this side, with the result that the stump will curve in a direction away from the block. The degree of curvature is directly proportional to the concentration of auxin in the block.

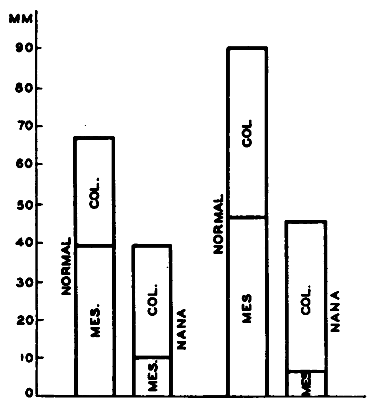


FIGURE 1

Length of 5-day (left-hand set) and 6-day old seedlings, showing the length of coleoptiles and mesocotyls separately.

If a series of tips of nana and another series of normal are placed on agar blocks (see scheme Fig. 2), then, after a certain time, the agar blocks from the normals contain more auxin than those from the nanas. This is always the case, whether the tips are long or short (see table 4) or the time of extraction is longer or shorter. Even after replacing the same tips 7 times on new agar blocks, the nana always give off less growth substance than the normal tips under similar conditions. The average ratio can be seen from figure 2. This means that if the amount of auxin given off by nana is 10, then the amount given off by normal will be 18.

3. *Less Growth with a Given Amount of Auxin in Nana Than in Normal.*—From corn seedlings the tips were removed and the remaining coleoptile cut off and stuck into very wet sand. After 2 or 3 hours, when these coleoptile stumps had lost their auxin, a new cut surface was made at the apical end, and blocks of agar containing auxin were put on one side. From the amount of curvature conclusions can be drawn about the relative growth (see Van Overbeek, 1933, Fig. 24, p. 594). If the nana and normal coleoptiles are treated in this way, then the nanas appear to curve less than the normals. The ratio can be seen from figure 3, and is as 10 in nana to 18 in normal. This is the same ratio that we have seen for the inhibition of production of auxin, which suggests that there must be a close relation between the two phenomena. This was found to be due to a *higher destruction of auxin in nana, causing both the decrease in amount of auxin given off by nana tips and the smaller growth with a given amount of auxin in nana.*

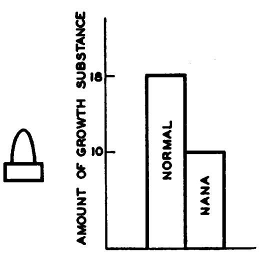


FIGURE 2

Relative amounts of growth substance given off by coleoptile tips of nana and normal. Average values of 400 tips each.

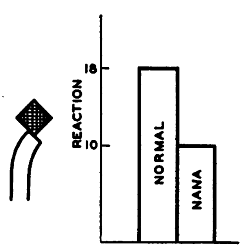


FIGURE 3

Relative curvatures with a given amount of growth substance applied one sidedly to nana and normal coleoptile stumps. Average of 200 plants each.

4. *Destruction of Auxin Higher in Nana Than in Normal.*—The destruction of auxin can be demonstrated in the following way. If the inhibition of growth is considered in relation to the destruction of auxin a great destruction will be expected in those parts of plants with a greatly inhibited growth. One of these parts is the mesocotyl of nana. If cylinders cut out of these mesocotyls are placed with their *basal* cut surface on blocks of agar containing auxin, the results after one or more hours should show whether growth substance has disappeared from the blocks. Using cylinders of 4 or 5 mm. cut from the apical ends of mesocotyls of normal and nana, the resulting destruction is found to be much greater in nana. Hetero-auxin[2] and auxin are both destroyed. The possibility that the growth substance is not destroyed but merely transported against the direction of the polarity was disproved as follows. To the basal cut surface of mesocotyl cylinders 5 mm. long, 20,000 units of hetero-auxin was applied, and no growth substance could be found in blocks of plain agar at the apical cut surface.

These experiments show the destruction of auxin clearly, but they do not prove it to be the cause of the growth inhibition in nana. The basal parts of normal mesocotyls appeared to destroy more auxin than the apical parts. Table 1 shows the destruction of auxin by mesocotyl cylinders 5 mm. long cut from the apical and basal part of the normals and from the apical part of nana.

TABLE 1

DESTRUCTION OF GROWTH SUBSTANCE BY MESOCOTYL CYLINDERS

| EXP. NUMBER | AMOUNT OF GR.S. LEFT NANA APICAL | NORMAL APICAL | NORMAL BASAL | AMOUNT OF GR.S. STARTED WITH | | DURATION OF EXPERIMENT | AGE OF PLANTS |
|---|---|---|---|---|---|---|---|
| 41026 | 4[1] | 11[1] | 2[1] | Auxin a | 11 | 3 hours | 5 days |
| 41201 | 2 | 7 | 2 | Hetero- } | 9 | 1 | 5 |
| | 2 | 7 | .. | auxin } | 9 | 1 | 4 |
| 41204 | 8 | 15 | 6 | H-a | 16 | | 4 |

[1] All figures in the tables are mean values of 12 tips, cylinders, plants, etc.

To separate the results and causes of the growth inhibition, the destruction of growth substance in coleoptiles was investigated. As mentioned above the length of coleoptiles is the same in nana and normal. If there can be found a difference in destruction in coleoptiles, it certainly is *not* a *result* of a difference in growth. There was difficulty in getting coleoptiles and especially the tips free from auxin. This was found to be possible, however, by placing them for about 20 hours on very wet filter paper. After this time the tips do not give off auxin any more, but if placed on blocks of agar containing auxin they destroy it, as is shown in table 2.

TABLE 2

DESTRUCTION OF GROWTH SUBSTANCE BY COLEOPTILE TIPS OF 5 MM.

| EXP. NUMBER | AMOUNT OF GR.S. LEFT. NANA | NORMAL | AMOUNT OF GR. S. STARTED WITH | DURATION OF EXPERIMENT | AGE OF PLANTS |
|---|---|---|---|---|---|
| 50228 | 4.5 | 8.0 | H-a 11.5 | 1 hour | 5 days |
| 50306 | 5.0 | 7.0 | H-a 14.0 | $1^1/_4$ | 5 |
| 50308 | 3.6 | 6.0 | H-a 7.5 | 1 | 5 |
| | 4.4 | 6.8 | H-a 7.5 | 1 | 5 |
| Total | 17.5 | 27.8 | 40.5 | | |
| Destroyed | 23 | 13 | | | |
| Ratio | 18 | 10 | | | |

The ratio of destruction in nana and normal tips is 10 to 18. We have seen before that the ratio of amount of auxin given off by these tips was 10 to 18. This makes it extremely likely that *the reduced amount of growth substance given off by nana can be explained by a higher destruction of growth substance in the dwarf.*

For coleoptile cylinders, cut from 5 to 10 mm. under the extreme tip, and freed of auxin, values were found as given in table 3.

These experiments show that in the region of nana coleoptiles which responds less to a given amount of auxin (see paragraph 3), the destruction of auxin is higher. The ratio of growth was 10 in nana to 18 in normal, the ratio of destruction is 16 in nana to 10 in normal.

From experiments in this paragraph, paragraph 8 and from experiments not yet published, it is clear that *the higher destruction of growth substance in nana is the cause of the dwarfed growth in nana.*

5. *In Coleoptiles Growth Substance Is Not the Limiting Factor.*—How is it, that in nana the length of the coleoptiles is the same as in normals,

TABLE 3

DESTRUCTION OF GROWTH SUBSTANCE BY COLEOPTILE CYLINDERS

| EXP. NUMBER | AMOUNT OF GR.S. LEFT NANA | NORMAL | AMOUNT OF GR.S. STARTED WITH | DURATION OF EXPERIMENT | AGE OF PLANTS |
|---|---|---|---|---|---|
| 50226 | 4.0 | 7.0 | H-a 9.0 | $3^1/_2$ hours | 5 days |
| 50306 | 5.7 | 8.4 | H-a 14.0 | $1^1/_4$ | 5 |
| | 7.0 | 9.3 | H-a 14.0 | $1^1/_4$ | 5 |
| 50308 | 2.5 | 4.0 | H-a 7.5 | 1 | 5 |
| | 1.8 | 3.7 | H-a 7.5 | 1 | 5 |
| Total | 21.0 | 32.4 | 52 | | |
| Destroyed | 31 | 19.6 | | | |
| Ratio | 16 | 10 | | | |

though in nana coleoptiles more growth substance is destroyed than in normal ones? Growth is dependent not only on auxin, but on other factors too. The factor whose relative amount is least will limit the growth. Thus Went pointed out (1928), that in the basal parts of the coleoptile and mesocotyl of Avena, auxin is a limiting factor and therefore regulates the growth in these parts. In the upper parts of coleoptiles, however, another factor is limiting (cell extension material). Now the fact that nana destroys more auxin than normal and still has the same length of coleoptile can easily be explained by assuming that auxin is present in such large quantities that it is still not a limiting factor, though some of it is destroyed in nana. That an excess of auxin is present in the coleoptiles can be shown (table 4), which gives the amounts of auxin extracted from long and short coleoptile tips during 3 periods of $1^1/_2$ hours in succession. During the first period the tips 2 mm. long give off about the same amount as tips 20 mm. long. If these tips are replaced on new agar blocks, the long tips produce less than the short ones.

The growth of the mesocotyl is quite dependent on the amount of auxin left by the coleoptile. If this amount is large, a large growth of the mesocotyl will result; if the amount is small, then little mesocotyl growth will result. This can be shown very nicely by decapitation experiments of normal plants. The amount of auxin produced by a new "physiological tip," if a tip of about 1 cm. is cut off, is considerably less than the amount produced by the original tip. Still this amount is sufficient for the coleoptile stump to grow as much as a comparable zone in the intact plant, but the growth of the mesocotyl is stopped completely. The amount of auxin left by the coleoptile is less in nana than in normal, because in nana more auxin is destroyed. The result will be a smaller amount of auxin available for the mesocotyl in nana, with a resulting smaller growth. This, together with the greater destruction of growth substance in the nana mesocotyl itself (paragraphs 4 and 6), is responsible for the smaller growth of the nana mesocotyls.

TABLE 4

AMOUNT OF GROWTH SUBSTANCE GIVEN OFF BY COLEOPTILE TIPS

Exp. number, 41024; plants, 5 days old; periods, $1^1/_2$ hours

| | LENGTH OF TIPS | FIRST PERIOD | SECOND PERIOD | THIRD PERIOD |
|---|---|---|---|---|
| Nana | 2 mm. | 11.0 | 13.5 | 15.9 |
| | 20 mm. | 11.6 | 9.0 | 9.8 |
| Normal | 2 mm. | 18.6 | 17.1 | 19.0 |
| | 20 mm. | 23.0 | 10.0 | 13.5 |

The *topmost internode* of corn plants grown under good conditions in the field is of the same length in normals and nanas (see photograph in Li, 1933). This can be explained in the same way as above for coleoptiles. It is a known fact that young flowers produce much growth substance. Very likely in young corn flowers so much auxin is produced that even if there is auxin destroyed in nana, the amount left is still not limiting for the growth in the internode below the flowers.

6. *Changed Oxidation-Reduction Properties in Nana?*—In what way is the auxin destroyed? Thimann (1934) tried to explain the fact that auxin in extracts is destroyed, by assuming that it is oxidized by enzymes. The enzymic activity of the two races of corn were therefore compared, and a higher catalase activity in nana was found (oxidase tests were negative). A higher catalase activity often indicates a higher peroxidase activity and changed oxidation-reduction properties. Cylinders of coleoptiles, mesocotyls and coleoptile tips of nana and normal were put into 5 cc. of a 0.03% pure $H_2O_2$ solution and shaken in an electric shaker in the dark room. After one hour 4 cc. of the solution was titrated with $KMnO_4$. In this way the amount of $H_2O_2$ destroyed could be determined. In coleoptile tips the catalase activity appeared to be higher in nana than in normal. In coleoptile cylinders and also in mesocotyl cylinders the same was found. A difference between tip and base in normal mesocotyls, however, could not be found. Table 5 gives some of the results.

TABLE 5

CATALASE ACTIVITY IN NANA AND NORMAL (AMOUNT OF $H_2O_2$ DESTROYED, IN 0.1 CC. OF $KMnO_4$)

In exp. 50122—15.5 cc. $KMnO_4$ = 4 cc. 0.03% $H_2O_2$
In exp. 50123—10.7 cc. $KMnO_4$ = 4 cc. 0.03% $H_2O_2$

| EXP. NUMBER | COLEOPTILE TIPS | | COLEOPTILE CYLINDERS | | MESOCOTYL TIPS | | MESOC. BASE | HOURS AFTER CUTTING OFF CYLINDERS OR TIPS |
|---|---|---|---|---|---|---|---|---|
| | NORMAL | NANA | NORMAL | NANA | NORMAL | NANA | NORMAL | |
| 50122 | 38 | 43 | .. | .. | 83 | 110 | 75 | 0–1 |
| | 22 | 39 | .. | .. | 54 | 76 | 54 | 1–2 |
| | 16 | 30 | .. | .. | 43 | 67 | 43 | 2–3 |
| | 14 | 29 | .. | .. | 36 | 60 | 36 | 3–4 |
| | 9 | 19 | .. | .. | 27 | 44 | 22 | 4–5 |
| 50123 | 14 | 21 | 29 | 51 | 31 | 52 | 31 | 1–2 |
| | 7 | 10 | 13 | 30 | 19 | 30 | 19 | 3–4 |
| Ratio | 10 | 16 | 10 | 19 | 10 | 15 | | |

The ratio of catalase activity in nana and normal is therefore between 15 and 19 in nana to 10 in normal, which certainly is of the same order as the ratios found for destruction of growth substance.

The *peroxidase*[3] activity was next compared in the two races. This was done by placing cylinders of nana or normal on agar blocks containing benzidine and $H_2O_2$. The more peroxidase, the more the benzidine is oxidized and the darker the color in the blocks. *The peroxidase activity appeared to be higher in nana than in normal,* and higher in the normal mesocotyl base than in the mesocotyl tip. In case of the higher peroxidase activity there are two possibilities: either the peroxidase oxidizes the auxin or it is merely an indicator of changed situations. If indeed the system peroxidase-peroxide is established, it is very likely that auxin is oxidized by it, since this system can oxidize compounds of the most varied types (see Onslow, 1921, p. 111). One thing seems possible, however, namely that in nana and normal the "oxidation levels" (r$H$?) are unlike.

7. *Making "Nana" from Normal by Heat Treatment.*—In one of his articles Von Euler (1919) describes how in yeast the catalase activity can be increased merely by raising the temperature to about 60°C. for one hour. If we can do the same with corn, and if an increase in catalase activity indicates a change in oxidation-reduction properties, and these in turn have something to do with the destruction of growth substance, we may expect normal plants after heat treatment to give plants of the type of nana. This was indeed found and not only this but the whole chain of differences as described above for nana and normal were found the same for treated and untreated normal plants. Normal plants were kept for one hour at a temperature of 45–50°C. in air. Six hours after treatment the *catalase activity* was 42 and 38 in treated coleoptile cylinders, against 23, 26 and 20 in untreated ones. Two hours after treatment the

*amount of auxin given off* by coleoptile tips was 3° in treated against 14° in the untreated ones. The *destruction* of auxin by mesocotyl tips was 3.5° in treated—$2^1/_2$ hour after treatment—against 0 in untreated. One day after treatment the treated destroyed 8° and the untreated only 1° from the 11° hetero-auxin started with. The *length* $1^1/_2$ days after treatment was 46 mm. in treated and 85 mm. in the untreated. *The phenomenon mentioned here certainly is an important support for the theory, that the dwarf type of growth of nana is due to an increased destruction of growth substance.*

8. *Summary.*—The causes of the dwarf type of growth of nana corn were studied. In nana more growth substance is destroyed than in normal, causing the inhibition of growth in the dwarf. The higher destruction of growth substance in turn may be due to a change in oxidation-reduction properties of nana.

[1] See Lindstrom (1923), Li (1933).

[2] Hetero-auxin appeared to be β-indolyl acetic acid, $C_{10}H_9O_2N$. Auxin a does not contain N, its formula is $C_{18}H_{32}O_5$ (see Kögl, 1935).

[3] *Catalase*, a haemin compound, reduces $H_2O_2$ to water and molecular oxygen. *Peroxidase*, also a haemin compound, reduces $H_2O_2$ and other peroxides only in presence of oxidizable compounds such as benzidine (and auxin). The oxygen from the $H_2O_2$ is "active" and carried from the peroxide toward the oxidizable substance. The optimum activity is found in a $H_2O_2$ concentration of 1:400.000.

Euler, H. von, and Blix, R., *Hoppe-Seyler*, **104**, 83 (1919).

Kögl, F., *Berichte deutschen chem. ges.*, **68**, Abt. A, 16 (1935).

Li, H. W., *Jour. Hered.*, **24**, 279 (1933).

Lindstrom, E. W., *Genetica*, **5**, 327 (1923).

Onslow, M. W., *Biochem. Jour.*, **15**, 107 (1921).

Thimann, K. V., *Jour. gen. phys.*, **18**, 23 (1934).

Van der Wey, H. G., *Proc. Amsterdam*, **34**, 875 (1931).

Van Overbeek, J., *Recueil des trav. bot. néerlandais*, **30**, 537 (1933).

Went, F. W., *Rec. des trav. bot. néerlandais*, **25**, 1 (1928).